REVIEW ARTICLE

Non-alcoholic Steatohepatitis: An Overview

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Non-alcoholic fatty liver disease (NAFLD) includes a broad spectrum of fat-induced liver injury, ranging from mild steatosis to cirrhosis and liver failure. The presence of obesity and insulin resistance is strongly associated with non-alcoholic fatty liver and a greater risk of advanced disease. We present here a review of the mechanisms involved in the pathogenesis of NAFLD, advances in the diagnosis, and options for treatment. [*J Formos Med Assoc* 2009;108(1):4–12]

Key Words: fatty liver, metabolic syndrome, steatohepatitis, steatosis

Non-alcoholic fatty liver disease (NAFLD), deposition of fat in the liver due to causes other than alcohol, includes non-alcoholic steatohepatitis (NASH). NASH is a more severe form of NAFLD in which fatty infiltration of the liver is accompanied by necroinflammatory activity, and is now recognized as one of the most common causes of chronic liver disease. Given the increasing prevalence of obesity worldwide, the deleterious effects of NAFLD, and more particularly NASH, are becoming of increasing concern for physicians.

Epidemiology

In 2003, it was estimated that the prevalence of NAFLD ranged from 17% to 33% in the general population of Western countries, and up to 80% in the morbidly obese population.[1] A study evaluating the prevalence of NASH estimated that 5.7–17% of the US population is affected.[2] It is reasonable to assume that these figures will continue to rise as the prevalence of obesity in the US becomes increasingly common. Less information is available on the prevalence of NASH beyond the Western world. There have been a few studies evaluating the prevalence and significance of NASH in East Asian populations. Chen et al evaluated a cross-sectional study of adults in rural Taiwan and found that the prevalence of NAFLD was 11.5%. Risk factors with development of NAFLD identified in this population were male sex, elevated alanine aminotransferase (ALT), obesity, elevated fasting plasma glucose (> 126), elevated triglyceride (> 150), and hyperuricemia.[3] Yui and Leung performed an epidemiologic study evaluating Hong Kong Chinese adults for NAFLD using ultrasound criteria and found that the overall prevalence of NAFLD in this population was 15.9%.[4] Fan et al evaluated the prevalence of NAFLD in the Shanghai adult male population and found similar results, with 20.82% of the population affected.[5] Studies of the Korean population revealed similar results, with an age-adjusted prevalence of 16%.[6] See the Table for a comparison of the prevalence of NAFLD in various populations.[3–7] Further studies are necessary to evaluate the significance of NAFLD and

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the prevalence and significance of NASH in these populations.

Natural History

The occurrence of NASH is clinically important as approximately 15–25% of these patients will progress to cirrhosis.[8–10] Once cirrhosis is present, it is estimated that 30–40% of these patients will progress to a liver-related death over a 10-year period.[8–10] These figures confirm the need to identify patients at risk, diagnose the presence of NAFLD and NASH at earlier stages in the clinical course, and intervene with patient education and therapeutic options. It is important to note, however, that only a small percentage (approximately 20%) of patients who develop NAFLD will go on to develop NASH.[2]

There is a clear correlation between NAFLD and NASH with obesity. As mentioned above, studies have shown that NAFLD is present in up to 80% of the morbidly obese population.[1] Additionally, there is an association between NAFLD and the metabolic syndrome. Some researchers have suggested that NAFLD, including NASH, may be the liver manifestation of the metabolic syndrome. Studies have shown a correlation between NAFLD and type 2 diabetes or impaired glucose tolerance.[11,12] This correlation may be important for identifying patients who are at increased risk of developing NAFLD including NASH.

Long-term data suggest that approximately 15–25% of patients who develop NASH will progress to develop liver cirrhosis.[8–10] However, this figure may be an underestimate. Several authors have proposed that many patients who are diagnosed with cryptogenic cirrhosis actually may have NAFLD- and NASH-associated cirrhosis. But, there is no evidence in the literature to suggest that patients with steatosis develop cirrhosis without first progressing to NASH, although there are reported cases of this phenomenon occurring in patients with alcoholic liver disease.[13]

Table. Comparison of the prevalence of non-alcoholic fatty liver disease in various populations

Population	Study size	Prevalence
Rural Taiwan[3]	3245	11.5%*
Hong Kong Chinese[4]	1627	15.9%*
Shanghai males[5]	3175	20.8%*
Korea[6]	1613	16.0%*
US Caucasians[7]	731	33.0%†
US Hispanics[7]	400	45.0%†
US African-Americans[7]	1097	24.0%†

**Evidence for NAFLD based on ultrasound data; †evidence for NAFLD based on proton magnetic resonance spectroscopy data.*

Pathophysiology

The development of non-alcoholic steatohepatitis is a complex process and is not completely understood. It has been suggested that the development of NASH is a two step process. The initial step involves deposition of fat in the liver (NAFLD), likely as a result of insulin resistance and increased fat mass. The second step involves oxidative stress and oxidation of the fat in the liver due to cytokine injury, hyperinsulinemia, hepatic iron and/or lipid peroxidation (Figure).[14] The development of insulin resistance is an intricate process. In the setting of the metabolic

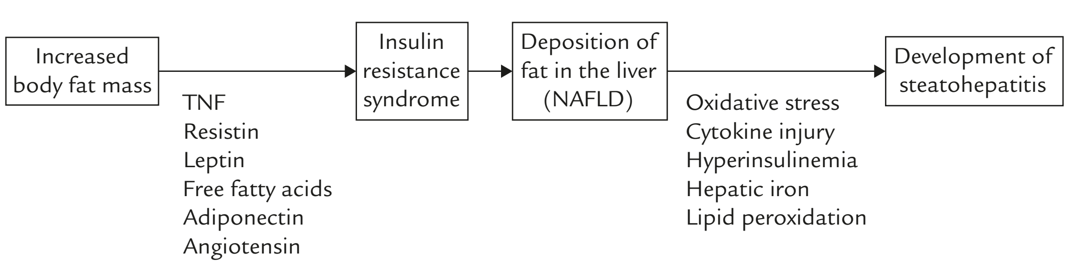

Figure. Scheme for the pathogenesis of steatosis and steatohepatitis.

syndrome, as is the case for many patients with NASH, the increase in fat mass and adipocyte differentiation plays a key role in the development of insulin resistance. This is achieved mainly by the adipocyte acting as an endocrine organ, secreting adipokines, which have various local and systemic responses. These include initiation of an acute phase response, alterations of lipoprotein metabolism, and energy homeostasis, variation of the extracellular matrix, and changes in the vasculature and immune system function.[15] These adipokines include tumor necrosis factor (TNF), adiponectin, leptin, resistin, and angiotensin. There have also been several studies evaluating the actions of various adipokines in patients with non-alcoholic steatohepatitis. It has been shown that TNF is increased in patients who are obese and have NAFLD.[16] In the setting of the metabolic syndrome involving the liver, the suspected mechanism of TNF involves induction of hepatic resistance to insulin via upregulation of the suppressor of cytokine signaling proteins by decreasing the ability of insulin to activate its signaling pathway resistance.[17–20] Additionally, adiponectin has been found to play a key role in insulin sensitivity,[21] and low levels have been shown to precede and be predictive of the onset of type 2 diabetes mellitus.[22] In NAFLD, adiponectin levels were found to be decreased.[23–25] Furthermore, it has been found that exogenously administered adiponectin in animal models of NAFLD improved hepatic steatosis.[26] With regards to angiotensin, the renin-angiotensin system has been shown to play a major role in the pathogenesis of hepatic fibrosis.[27,28] Early research has shown that angiotensin II antagonists may halt the progression of fibrosis in animal models,[29] and may reverse NAFLD-induced elevations of liver function tests in humans.[30]

Insulin resistance ultimately results in hyperinsulinemia with an increase in free fatty acid (FFA) flux to the liver. Over a period of time, this leads to the development of hepatic steatosis and NAFLD. It is unclear why some patients who develop NAFLD go on to develop NASH while others do not. As mentioned above, it is suspected that some type of oxidative stress is involved. There are essentially four potential mechanisms that are suspected to cause this oxidative stress. The first mechanism is the production of proinflammatory cytokines by Kupffer cells causing mitochondrial oxidative stress of hepatocytes, and ultimately leading to necrosis and apoptosis. This pathway was explored and detailed in animal models.[31] The second proposed method of oxidative stress development is through hyperinsulinemia itself. It is thought that the key to insulin's ability to cause oxidative stress lies in its ability to upregulate the lipogenic protein, SREBP,[32] as well as its direct profibrogenic effects through stimulation of connective tissue growth factor.[33] The third proposed mechanism is iron overload generating reactive oxygen species and subsequent lipid peroxidation. Iron is known to have damaging effects on mitochondria,[34] and approximately 30% of NAFLD patients do have elevated ferritin levels.[35,36] Additionally, phlebotomy has been shown to improve liver histology in patients with NAFLD.[37] The last proposed mechanism of oxidative stress in NASH involves lipid peroxidation of FFAs. Patients with NAFLD have increased delivery of FFAs to the liver[38] where oxidation of the FFAs produces hydrogen peroxide, superoxide and lipid peroxides, all of which generate oxidative stress.

Diagnosis

The gold standard for diagnosis of NAFLD including NASH is the liver biopsy. The National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored NASH Clinical Research Network (CRN) has developed a histological scoring system which is uniformly used for all clinical trials involving NASH. Three distinct histologic lesions are necessary to make the diagnosis of NASH. These are zone 3 macrosteatosis, hepatocyte ballooning and mixed lobular inflammation. There are other common findings on liver biopsy in patients with NASH, including portal inflammation, perisinusoidal zone 3 fibrosis, acidophil bodies, lipogranulomas, glycogenated nuclei, periodic acid staining positive (after diastase) Kupffer cells, Mallory's hyaline, megamitochondria and mild siderosis.[39] The severity of disease is determined by the *NAFLD activity score* (NAS) and the *fibrosis score*. These gradations are useful for assessing change during clinical trials. The major limitation of liver biopsy is sampling variability, which may lead to misdiagnosis and staging inaccuracies. Several studies have shown sampling variability and uneven distribution of histologic lesions of NASH during evaluation of paired biopsies.[40–42]

Although liver biopsy is the current "gold standard" for diagnosis of NAFLD including NASH, it is not a practical screening tool given the cost, time-intensive nature and potential morbidity of this procedure. Multiple modes of imaging can be utilized to evaluate patients for NASH to varying degrees of effectiveness. Ultrasound is a relatively inexpensive form of liver imaging. Ultrasound showing hyperechogenic liver tissue in contrast to the spleen or kidney echogenicity is suggestive of steatosis. However, the sensitivity of ultrasound is only 60–94%.[43] In addition, ultrasound is not able to stage liver fibrosis. Unenhanced computed tomography (CT) shows low liver attenuation in steatosis, and the severity of steatosis correlates with the liver-to-spleen attenuation ratio.[44,45] The sensitivity of unenhanced CT is 93%, with a 76% positive predictive value in patients with greater than 33% steatosis.[46] Gradient-echo magnetic resonance imaging is also useful to evaluate liver steatosis, with a sensitivity of 80% and specificity of 71% in patients with at least 10% steatosis.[47]

A current area of research is the development of biomarkers to determine the extent of fibrosis. Suzuki et al evaluated hyaluronic acid as a predictor of fibrosis in 79 patients, and found that it was a reliable predictor in patients with grade 3–4 fibrosis (area under the curve, 0.90). However, it was not sensitive for detecting mild fibrosis.[48] Endothelin-1 is another mediator of hepatic fibrosis that has been evaluated as a predictor for fibrosis. Degertekin et al evaluated 40 patients with biopsy-proven NASH and found that average endothelin-1 levels were significantly higher when compared to patients with simple steatosis.[49]

Several diagnostic panels have been created to predict fibrosis scores in patients using a variety of clinical data. The NAFLD fibrosis score is a panel using age, body mass index, platelet count, albumin, aspartate aminotransferase (AST)/ALT ratio and hyperglycemia to predict fibrosis. It was validated in two studies with 480 patients and 253 patients, respectively, and found to reliably predict fibrosis with a negative predictive value of 93% and 88%, and a positive predictive value of 90% and 82%.[50] A limitation of this panel is that approximately 25% of patients are classified as "indeterminate". The *Original European Liver Fibrosis* test is a panel utilizing similar markers to the NAFLD fibrosis score in addition to tissue inhibitor of metalloproteinase 1 (TIMP 1), hyaluronic acid, and P3NP (procollagen-3 N-terminal peptide). It was validated in a study with 196 patients, and found to have an area under the curve of 0.98 for severe fibrosis, 0.93 for moderate fibrosis, and 0.84 for no fibrosis.[51] The relative accuracy of these two diagnostic panels suggests that prediction of liver fibrosis is possible using a series of markers. However, further studies with larger sample sizes are needed to confirm their accuracy.

Treatment

As mentioned above, obesity is one of the most commonly associated comorbid conditions with NASH. Several studies have suggested that weight loss has beneficial effects on improvement of, and potential reversal of NAFLD and NASH. The majority of studies focused on improvements in liver biochemistry profiles in response to weight loss. Palmer and Schaffner demonstrated that a loss of at least 10% of body mass in 39 obese patients was associated with reversal of abnormal liver function tests as well as decreased hepatomegaly.[52] A study of 25 obese Japanese patients showed improvement in aminotransferases as well as total cholesterol and fasting glucose levels in the subset of patients who underwent a 3-month program of dietary restriction and exercise.[53] Knobler et al studied 48 patients who underwent dietary restriction with resultant moderate weight loss (mean 3.7 kg weight loss at 24-month follow-up) and found that 96% of patients had improvement in liver function tests and 50% had complete reversal of previously elevated transaminases.[54]

Regarding pharmaceutical interventions, several studies have investigated the effects of insulin sensitizers on the improvement of NASH, given the large percentage of patients with NASH who have coexisting insulin resistance. Rosiglitazone (4 mg BID for 48 weeks) was shown to significantly improve insulin sensitivity and serum ALT levels in 25 patients with biopsy-proven NASH, and repeat biopsy after 11 months of treatment revealed histologic improvement in necroinflammatory score in 45% of these patients. Unfortunately, further follow-up at 6 months after treatment cessation showed elevation of liver enzymes to near pretreatment levels.[55] Pioglitazone (30 mg daily for 48 weeks) has also been shown to improve insulin sensitivity, serum ALT levels as well as liver histology. A reduction in fibrosis score on liver biopsy after 48 weeks of therapy was seen in 51% of patients in this study. Interestingly, follow-up data in patients 48 weeks after stopping therapy showed a significant increase in serum ALT, and worsening of all histologic findings except fibrosis score to pretreatment values.[56] A second study by Belfort et al confirmed these findings. Fifty-five patients with biopsy-confirmed NASH were randomized and given pioglitazone (45 mg daily) versus placebo along with a hypocaloric diet. The pioglitazone group was found to have a significantly higher level of normalization of serum aminotransferase levels, decreased hepatic fat content, and improvement of histologic findings.[57] Some major drawbacks to the use of the above thiazolidinediones are the potential risk of hepatotoxicity and their contraindication for use in patients with active liver disease with serum ALT levels more than 2.5 times the upper limit. There have been two reports of hepatic dysfunction associated with troglitazone. Gitlin et al reported two patients with underlying diabetes and obesity, but no known baseline liver disease who developed severe hepatotoxicity after troglitazone therapy.[58] Watkins and Whitcomb reviewed all clinical trials involving troglitazone, and found that of the 2510 patients involved in studies, 1.9% developed elevations in serum ALT concentrations to greater than three times the normal limit.[59]

Metformin has also been widely studied in the setting of NASH. A study evaluating the effect of metformin (850 mg BID for 24 weeks) in 36 patients with biopsy-proven NASH showed that insulin sensitivity, and serum ALT and AST improved significantly in the treatment group. However, histologic evaluation in this study failed to show significant improvement of necroinflammatory activity or fibrosis score.[60] In a second controlled study by Bugianesi et al, 55 patients with NASH were given metformin (2 g daily for 48 weeks) and were found to have improvements in serum ALT levels and decreased prevalence of metabolic syndrome when compared to control groups after 48 weeks of therapy. Histologic evaluation showed significant decreases in necroinflammation and fibrosis in the treatment group after completion of 48 weeks of treatment. However, no biopsies were taken of the control group for comparison.[61]

Attention has also been directed to the role of antioxidants in the treatment of NASH, given the proposed theory of its pathogenesis. A small study by Hasegawa et al of 12 patients evaluated the effect of vitamin E (300 mg/day for 1 year) on transaminase levels and liver histology. Liver biopsy following 1 year of treatment showed improvement in inflammation and fibrosis as well as improvement in serum ALT levels.[62] Other studies, however, have shown no beneficial effects. Kugelmas et al evaluated the effects of vitamin E (800 IU daily for 6 weeks) in a controlled study of 16 patients with biopsy-proven NASH, and found no significant improvement in transaminase levels.[63] Larger randomized controlled studies are needed to further evaluate the effects of antioxidants for this condition.

Ursodeoxycholic acid (UDCA) has also been evaluated as a potential therapy for NASH. In a smaller study by Lindor et al, 126 patients with biopsy-proven NASH were randomized to receive between 13 and 15 mg/kg/day of UDCA for 2 years versus placebo. There were no significant changes in the degree of steatosis, necroinflammation, or fibrosis on repeat liver biopsy found in the treatment group.[64]

The role of antihyperlipidemic agents in the setting of NASH has also been analyzed in several small studies. Gemfibrozil (600 mg daily for 4 weeks) was evaluated in 46 patients with biopsy-proven NASH, and found to improve serum ALT levels in patients after 4 weeks of treatment when compared to a control group; however, no histologic improvement was seen on repeat liver biopsy.[65] A small study of six patients evaluating clofibrate (2 g daily for 12 months) versus control group found that clofibrate did not improve ALT levels or liver histology on evaluation after 1 year of treatment.[66] Statins have been shown to reduce ALT levels;[67,68] but data are limited with this drug class given its potential risk of hepatotoxicity. A small study of five patients evaluated the effects of pravastatin (20 mg daily for 6 months) and found improvement of fibrosis scores in three of the five patients after 6 months of treatment.[68]

Surgical options for weight loss are becoming increasingly utilized in the US health care system. Systematic reviews have shown that all procedures are able to achieve more than 50% loss of excess weight.[69–71] However, there are no randomized controlled trials to evaluate the effects of bariatric surgery on liver disease. There are several case series, and observational studies that do suggest improvement of steatosis and inflammation following surgically-induced weight loss. Mattar et al evaluated 70 patients with paired biopsies pre- and post-weight loss from gastric bypass surgery. Patients were re-evaluated on average 15 months postoperatively. The mean excess body weight loss at the time of re-evaluation was $59 \pm 22\%$. They noted significant improvement in liver steatosis, inflammation and fibrosis scores upon repeat biopsy.[72] Other smaller studies have confirmed these findings.[73,74] Gastric banding has also been shown in small studies to improve NAFLD and NASH. An evaluation of liver histology in 36 patients who underwent gastric banding showed major improvements in lobular steatosis, necroinflammatory changes and fibrosis after surgically induced weight loss (average weight loss, 34 kg).[75]

Conclusion

NASH is becoming an increasingly common disease process, currently affecting up to 17% of Western populations.[2] Given the potential complications of NASH, including cirrhosis, it is essential to identify patients at risk and direct early intervention. Currently, the gold standard for diagnosis is the liver biopsy; however, other diagnostic tools, including imaging and biomarkers, are being evaluated. The optimal treatment for NASH is weight loss and lifestyle modifications including diet and exercise. Surgical procedures for weight loss have also been shown to improve NASH. Metformin has also been shown to be a relatively safe pharmaceutical intervention to improve liver histology. Data suggest that significant lasting weight loss is the only therapeutic intervention that can lead to longstanding resolution of NASH.

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